Comparison of chicken breast quality characteristics and metabolites due to different rearing environments and refrigerated storage

Doo Yeon Jung,* Dongheon Lee,* Hyun Jung Lee,* Hee-Jin Kim,[†] Jong Hyun Jung [®],[‡] Aera Jang,[†] and Cheorun Jo [®]*,[§],[1]

*Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea; [†]Department of Applied Animal Science, College of Animal Life Science, Kangwon National University, Chuncheon 24341, Republic of Korea; [‡]Jung P&C Institute, Inc. Yongin 16950, Republic of Korea; and [§]Institute of Green Bio Science and Technology, Seoul National University, Pyeongchang 25354, Republic of Korea

ABSTRACT The objective of the present study was to compare the breast meat quality and metabolomic characteristics from broilers that were raised in conventional (conventional farm reared-broilers; **CB**, n = 20) and legally approved animal welfare farms (welfare farm reared-broilers; **WB**, n = 20) in aerobic cold storage (1, 3, 5, and 7 d). Compared to CB chickens, the WB chickens had a larger floor size as well as lower stocking density, atmospheric ammonia, and nipple-shared chicken counts. The results demonstrated significantly higher pH, L^*- and b^*-value, and lower shear force in CB compared to WB during cold storage. Using ^{1}H NMR analysis, 25 compounds were identified in the chicken breast meat. Partial least square-discriminant analysis (**PLS-DA**) was performed based on the identified metabolites. The content of 15 metabolites (1 di-peptide, 9 free amino acids, 2 glycolytic potential-related products, 2 nucleotide-related products, and 1 organic acid) was significantly different due to the rearing environment (CB vs. WB). Among them, all free amino acids were higher in CB than in WB. Six free amino acids (glycine, isoleucine, leucine, phenylalanine, valine, and β-alanine) had variable importance in projection (**VIP**) score >1, regardless of the number of cold storage days. Therefore, these compounds in the breast meat may be used as potential markers to determine the rearing environment of broilers. Also, this result might be an indication of stress-related meat quality changes in broilers.

Key words: chicken breast meat, animal welfare, meat quality, metabolomic analysis

INTRODUCTION

Chicken breast meat is recognized as a healthy food source with an excellent nutrient composition (da Silva et al., 2017). It is high in protein content and low in cholesterol and fat content, as well as low in calories (Kim et al., 2020). Therefore, in terms of nutrition, it is more attractive to the modern health-conscious consumer (Petracci et al., 2014). With the increasing trend in wellness-oriented consumerism, the consumption of chicken breast meat has increased along with the consumers' interest in improving meat quality such as texture, flavor, juiciness, appearance, health, organic, and safety (Henchion et al., 2014). The consumer's request for animal welfare during the meat production process has raised the question as to whether animal-friendly rearing has an impact on the meat quality (Enfält et al., 1997; Lin et al., 2014; da Silva et al., 2017).

During rearing, chickens can be exposed to various environmental factors (e.g., equipment and facilities, stocking density, and air quality), inducing different levels of stress (Muroya et al., 2020). In particular, broilers reared on high stocking density are exposed to heat stress and increased atmospheric ammonia with increased body temperature, resulting in a decrease in immunity and antioxidant defense system with increased reactive oxygen species (An et al., 2012). In the Republic of Korea, a certification system for animal welfare farms has been implemented since 2014 for broilers (MAFRA, 2012). Animal welfare farms can be approved if they have an animal-friendly breeding environment (e.g., stocking density, ammonia concentration, feeders, waterers, plant source, etc.) following the standards set by the Ministry of Agriculture, Food and Rural Affairs (MAFRA, 2012; Kim et al., 2020). Several studies have been reported

lower physiological stress levels in broilers reared on animal welfare farms approved in the Republic of Korea compared to conventional farms (Kim et al., 2021c,d). Stress can change the final chicken meat quality because stress-induced effects result in changes in the metabolic functions and biochemistry in postmortem muscles (Xing et al., 2019; Muroya et al., 2020). Many studies have been conducted to analyze the changes in the meat quality in different rearing environments controlled by various environmental factors (air quality, density, grazing, temperature, etc.), and the meat quality may differ depending on the environmental factors (Zhang et al., 2012; Kim et al., 2020; da Rosa et al., 2021). In fact, it has been confirmed in several studies that chicken meat quality is improved when broilers are reared in animal-friendly conditions (Castellini et al., 2002; da Silva et al., 2017; Kim et al., 2020). Meanwhile, it is still unclear how pre-slaughter environmental stressors affect chicken meat quality during extended storage period. The meat quality can be also changed during storage due to microbial growth, protein degradation, and lipid oxidation (Nychas et al., 2008; Jung et al., 2010; Fu et al., 2015; Wen et al., 2020).

Metabolomics is a rapidly developing field in meat science and involves the analysis of small compounds (<1,500 Da) to explain changes in meat quality characteristics under various physiological and environmental conditions (Wen et al., 2020). Kim et al. (2021a) reported the differences in chicken breast meat metabolites obtained from different strains and lines, using one-dimensional and two-dimensional quantitative nuclear magnetic resonance (**NMR**) spectroscopy and separated them using multivariate analysis. The metabolomic analysis can be an effective approach to understand the changes in chicken breast meat quality under different rearing conditions and cold storage (Muroya et al., 2020). In the present study, we implemented metabolomic analysis to compare the quality difference between breast meat from broilers reared in conventional (**CB**) and welfare farms (**WB**) over a 7-d cold storage period. This study provides the valuable basic data for explaining the changes in the metabolite profiles of chicken breast meat based on different rearing environment and extended storage effects. Furthermore, we suggest differences in meat quality based on major metabolites that distinguish WB from CB for consumers who prefer to purchase chicken breast reared in animal-friendly environments.

MATERIALS AND METHODS
Preslaughter Conditions and Meat Sampling

One-day-old 76,000 Cobb chicks (mixed in male and female) were reared in conventional or legally approved animal welfare farm for 35 d, respectively (Table 1). The atmospheric ammonia concentration was measured and monitored in real-time throughout the 35-d rearing period using ammonia meters (MiniMAX XP, Honeywell, Morristown, NJ) in both farms. All chicks were fed with crumble feed during the starter and grower periods,

Table 1. The rearing conditions of chicken breast meats from conventional and animal welfare farms.

Rearing conditions	Experimental group[1]	
	CB	WB
Floor size (m^2)	929	1,027
Stocking density (chicks/m^2)	25	17
Atmospheric ammonia (ppm)	50−100	<25
Number of nipples	1 per 13−15 chicks	1 per 10 chicks

[1]Abbreviations: CB, conventional farm reared-broilers; WB, welfare farm reared-broilers.

and pellet feed in the finisher period, following the animal welfare approved farm standards (MAFRA, 2012). The WB group was provided a non-animal-derived diet and other substances (perch, sawdust, rice straw, and plant sources) to meet physiological and pecking needs. For both groups, dietary metabolizable energy in the starter (0−7 d), grower (8−19 d), and finisher periods (20−35 d) was 3,090, 3,180, and 3,250 kcal/kg, respectively, and the crude protein values of each diet were 22.50, 20.20, and 19.20%, respectively.

After 35 d of rearing, the chickens were transported for 90 min to the slaughterhouse and held in a lairage for 4 to 6 h. Water was provided ad-libitum, and the fasting time were 5 h. The chickens were stunned using 63 to 80% CO_2 gas and slaughtered in strict accordance with Livestock Products Sanitary Control Act, Republic of Korea, and carcasses from CB (n = 20) and WB (n = 20) were randomly selected and purchased from a commercial slaughterhouse (Iksan, Korea). All efforts were made to minimize the suffering of the animals. Subsequently, the samples were transferred to a laboratory (Chuncheon, Korea) using a cooler with ice. Both sides of breast fillet (*M. pectoralis major*) were obtained, placed on a polystyrene tray and wrapped in low-density polyethylene (oxygen transmission rate = 35,273 cm^{-3} m^{-2} day^{-1} at p (O_2) = 1 atm). The chicken breast samples were stored for 7 d in a walk-in cooler at 4 ± 1°C and analyzed after 1, 3, 5, and 7 d of storage. The left side breast muscle was used to measure color, cooking loss, and shear force. The right side breast was prepared by pooled after grinding and then taken to analyze other meat quality traits and metabolomics. Samples were analyzed immediately on each storage day (1, 3, 5 and 7 d) or stored at −70°C until analysis.

Physicochemical Traits

pH Measurements The pH of the meat samples was measured using a pH meter (Orion 230A, Thermo Fisher Scientific, Inc., Waltham, MA) on all designated storage days (1, 3, 5, and 7 d). Briefly, 10 g of sample and 90 mL of distilled water were homogenized (PolyTron PT-2500 E, Kinematica, Lucerne, Switzerland), and the pH value of the homogenate was measured after calibration with standard buffers (4.01, 7.00, and 9.21).

Color After removing the skin, meat color was measured on the skin side of each breast fillet was measured using a colorimeter CR-400 instrument (Minolta Co.,

Osaka, Japan) with illuminant D65. The color values were expressed as Commission Internationale de l'Eclairage (**CIE**) color value of L^* (lightness), a^* (redness), and b^* (yellowness). The colorimeter was calibrated using white plate references (Y = 84.6, x = 0.3174, y = 0.3241).

Water-Holding Capacity and Cooking Loss The water-holding capacity (**WHC**) was calculated using the method described by Jang et al. (2011). Briefly, the breast sample (0.5 g), with connective tissue removed, was heated in a water bath (80°C, 20 min) and cooled to room temperature for 10 min. After centrifugation at $2,000 \times g$ for 20 min, water loss was measured. The WHC (%) was calculated using the water loss by centrifugation and moisture content.

For cooking loss analysis, left side breast meat was placed in a polyethylene bag and cooked in a water bath (75°C, 45 min) until the core temperature reached over 73 ± 2°C according to Kim et al. (2021e). The cooking loss of the samples was expressed as the percentage of weight loss before and after heating. The cooking loss was measured on all designated storage days.

Shear Force After cooking until the internal temperature reached 73 ± 2°C, the samples were cut into $3 \times 1 \times 2$ cm^3 (width $\times$ depth $\times$ height), and the shear force was analyzed using a TA1 texture analyzer (Lloyd Instruments, Fareham, UK) with a V blade (60° V-notch). The analyzer settings were as follows: 500 N load cell, 50 mm/min test speed, 50 mm/min trigger speed, and 0.1 N trigger force.

Storage Stability

Microbial Growth For determining microbial growth, the analysis was conducted on each storage day (1, 3, 5, and 7 d). The total counts of aerobic bacteria and coliforms were measured using 3M Petrifilm (Aerobic Count Plates, Coliform/*E.coli* count plates, 3M, Saint Paul, MN). The breast sample (10 g) was placed in a sterile bag with 90 mL of saline and homogenized for 1 min using a stomacher (BagMixer 400 P, Interscience, France). After serial dilution of the homogenate, 1 mL of dilution was plated on the 3M petrifilm and incubated for 48 h at 37°C. After incubation, the results were expressed as log CFU/g.

Lipid Oxidation and Volatile Basic Nitrogen Value Lipid oxidation (2-thiobarbituric acid-reactive substance [**TBARS**]) and Volatile Basic Nitrogen (**VBN**) values were analyzed to evaluate quality deterioration during storage (Lin and Lin, 2005). All analyses were conducted according to the methods described by Shin et al. (2021). Briefly, the TBARS value was determined by homogenizing 5 g of each sample with 15 mL of deionized distilled water and 7.2% butylated hydroxyl toluene. The homogenized mixture (1 mL) was transferred to new test tubes, and 2 mL of 20 mM 2-thiobarbituric acid in 15% trichloroacetic acid was added. The tubes were heated in a water bath for 15 min at 90°C, cooled, and centrifuged at $2,000 \times g$ for 10 min. The

absorbance of the supernatant was determined using a spectrophotometer (M2e, Molecular Devices, Sunnyvale, CA) at 531 nm. The TBARS value was expressed as mg malondialdehyde/kg breast meat as follows:

$$\text{TBARS (mg MDA/kg)} = (\text{absorbance of sample} - \text{absorbance of blank sample}) \times 5.58$$

The VBN value was determined by homogenizing 10 g of each sample with 50 mL of distilled water using a magnetic stirrer for 30 min. The homogenate was filtered through filter paper (Whatman No. 1, Whatman PLC., Kent, UK), and 1 mL of the filtrate was added to the outer chamber of a Conway micro-diffusion cell. Then, 1 mL of 0.01 N H_2SO_4 was placed in the inner cell, and saturated K_2CO_3 (1 mL) was added to the other outer cell. The cell was sealed immediately and incubated at 25°C for 1 h. Subsequently, 10 μL of the Brunswick reagent was placed in the inner section and titrated with 0.01 N NaOH. The VBN values were recorded as mg %.

1D ^{1}H NMR-Based Metabolites Polar metabolite extraction and 1D ^{1}H NMR analysis were performed according to the method described by Kim et al. (2019). Briefly, chicken breast samples were collected within 30 minutes at each storage day (1, 3, 5, and 7 d). The chicken breast sample (5 g) was extracted with 20 mL of 0.6 M perchloric acid. Then, the homogenate was centrifuged (Continent 512R, Hanil Co., Ltd., Daejeon, Korea) at $3,500 \times g$ for 20 min, and the supernatant was titrated to pH 7.0, using KOH. Subsequently, each extract was filtered using filter paper (Whatman No. 1, Whatman PLC.) and freeze-dried. The lyophilized sample was diluted in 20 mM phosphate buffer (pH 7.4) using deuterium oxide containing 1 mM 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid and used for NMR analysis. ^{1}H NMR spectra were measured using a Bruker 850 MHz cryo-NMR spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany). All spectra were analyzed using Topspin 4.0.8 (Bruker Biospin GmbH), and each peak was identified using Chenomx NMR suite 7.1 (Chenomx, Inc., Edmonton, AB, Canada) and Human Metabolome Database (www.hmdb.ca).

Statistical Analysis

All analyses for chicken breasts with different breeding environments were conducted in five replicates for each of 1, 3, 5, and 7 refrigerated storage days. The results of physicochemical traits and storage stability were evaluated with ANOVA, and significant differences between the mean values were verified using Tukey's multiple test in SAS 9.4 (SAS Institute Inc., Cary, NC) with a significance level of $P < 0.05$. For quantification results of metabolite analysis, the effects of different rearing conditions of the farm during cold storage on each metabolite were estimated using two-way ANOVA, and the identified metabolites were used to perform the partial least squares-discriminant analysis (**PLS-DA**) for multivariate analysis using MetaboAnalyst 4.0. All

samples were log-transformed and auto-scaled prior to analysis.

RESULTS AND DISCUSSION

Physicochemical Traits

Stress conditions have shown to affect the muscle pH, which in turn affects the overall meat quality (Zaboli et al., 2019). Table 2 shows the physicochemical traits of CB and WB in chilled storage for 1, 3, 5, and 7 d. The pH of WB was significantly lower than that of CB, except on d 7. The lower pH of WB may be attributed to the high glycogen content due to less stress under WB conditions (Ponte et al., 2008). Castellini et al. (2002) reported that the significantly lower pH in the meat of less stressed, organic broilers, compared to indoor-reared broilers, may be due to higher glycogen levels being stored in the organic broilers at slaughter, thereby inducing a further drop in the pH. Meanwhile, the pH of both CB and WB increased over the 7 d of cold storage ($P < 0.05$). The increased pH value of chicken breast meat may be associated with the generation of nitrogenous base compounds by microbial spoilage (Triki et al., 2018).

Meat color, which can be influenced by several physical and chemical factors, is an essential quality parameter that affects consumers' perceptions and selection of raw meat (Karunanayaka et al., 2016; Faustman and Suman, 2017). In the current study, the L^* value of CB was higher than that of WB after d 3 ($P < 0.05$). A rapid pH drop that is usually affected by pre-slaughter stress gives rise to a pale meat color (Barbut, 1993; Karunanayaka et al., 2016); however, our study showed conflicting results, which may be due to stress-induced muscle proteolysis in CB. As it is well reported, in stressful environments, muscle protein breakdown is accelerated due to secreting corticosterone (Zhang et al., 2012; Ma et al., 2021). This muscle proteolysis can generate the low-molecular weight peptides and amino acids, which increases light scattering and reflection of muscle by changing the refractive index (Hughes et al., 2014). Therefore, the higher L^* value in CB may be due to increasing light reflection and change of muscle structure by stressful rearing condition. During the 7 d of cold storage, the b^* value of CB was significantly higher than that of WB, whereas the a^* value showed a similar level. The possible reason for the higher b^* value in the breast meat from more stressed broilers is unclear, but presumably, it might be due to oxidative stress damaged biological macromolecules, such as proteins, lipids, and DNA (Zhang et al., 2011). Therefore, further studies that analyze the correlation between meat yellowness and oxidative damaged biological macromolecules should be conducted. Generally, yellow meat color is considered unpleasant because consumers believe that yellowish tissues originate from old, undernourished, or unhealthy animals (Troy and Kerry, 2010). The chicken breast meat from less stressed conditions (WB) is likely to be preferred by consumers due to the desirable meat color of WB. In terms of change in storage period, no difference between the L^* and a^* values of between the chicken breasts from the two environments was observed on most storage days, whereas the b^* value increased as the storage period progressed. According to Marcinkowska-Lesiak et al. (2016), the alteration of meat pigment, particularly due to the generation of metmyoglobin, leads to an increase in b^* value during cold storage. This may be one of the reasons that underpins the increasing b^* values of CB and WB during longer storage time.

Table 2. Physicochemical traits of chicken breast meat from conventional and animal welfare farms during cold storage.

Item	Treatment	Storage (days) 1	3	5	7	SEM[1]
pH	CB	5.93[A,b]	6.12[A,a]	6.15[A,a]	6.18[a]	0.037
	WB	5.72[B,c]	5.92[B,b]	6.00[B,ab]	6.11[a]	0.034
	SEM[2]	0.038	0.034	0.041	0.026	
CIE L^*	CB	50.51[b]	52.15[A,a]	52.15[A,a]	52.73[A,a]	0.286
	WB	51.11	50.70[B]	50.76[B]	50.41[B]	0.381
	SEM[2]	0.338	0.332	0.363	0.313	
CIE a^*	CB	1.76[A]	1.70	1.54	1.47	0.159
	WB	1.02[B]	1.23	1.17	1.10	0.114
	SEM[2]	0.081	0.198	0.130	0.119	
CIE b^*	CB	6.27[A,b]	6.64[A,ab]	7.47[A,a]	7.48[A,a]	0.242
	WB	3.75[B,b]	4.33[B,ab]	4.35[B,ab]	5.15[B,a]	0.275
	SEM[2]	0.300	0.265	0.277	0.177	
Cooking loss (%)	CB	17.95[b]	27.72[a]	26.73[a]	27.21[B,a]	1.749
	WB	18.46[b]	31.33[a]	28.60[a]	31.46[A,a]	1.143
	SEM[2]	0.568	2.446	1.229	0.956	
WHC (%)	CB	53.78	56.34	54.03	55.21	0.924
	WB	53.09	53.46	53.72	55.48	1.334
	SEM[2]	0.803	1.529	0.934	1.188	
Shear force (N)	CB	23.99[B,a]	21.57[B,ab]	20.40[B,bc]	18.81[B,c]	0.647
	WB	28.39[A,a]	24.83[A,ab]	24.45[A,ab]	21.59[A,b]	1.505
	SEM[2]	1.324	1.173	1.057	1.058	

Abbreviations: CB, conventional farm reared-broilers; WB, welfare farm reared-broilers; WHC, water holding capacity.
[A,B]Different letters within the same column indicate significant differences ($P < 0.05$).
[a-c]Different letters within the same row differ significantly ($P < 0.05$).
[1]Standard error of mean (n = 20).
[2]Standard error of mean (n = 10).

The WHC of meat can be influenced not only by external factors (e.g., storage, processing, and cooking) but also by intrinsic factors (e.g., animal gentetics, pre-slaughter stress, and postmortem condition) (Warner, 2017). Additionally, WHC can be expressed as cooking loss, which measures the weight loss of meat during cooking and may have a significant impact on the overall sensory attributes of the meat (Warner, 2017; Oswell et al., 2021). In this study, there was no significant difference in cooking loss and WHC between CB and WB (Table 2). Similarly, the moisture content was not significantly different (Table S1). During the storage period, the cooking loss and WHC of CB and WB did not change, except for cooking loss on d 3 ($P < 0.05$).

Meat tenderness is an important quality attribute that affects the meat palatability, and the shear force value is one of the most representative instrumental methods to measure it (Dodge and Stadelman, 1960). In this study, the shear force value of WB was higher than that of CB during all cold-storage times ($P < 0.05$; Table 2). In general, stress leads to a soft texture owing to a rapid pH drop (Kim et al., 2014); however, in this study, the stressful rearing environments had a greater effect than pH on the meat tenderness. CB broilers were exposed to a more stressful rearing condition and accelerated muscle proteolysis, resulting in increased meat tenderness. The shear force is negatively correlated with the proteolysis level of the myofibrillar protein, which is induced by several stressors (Zhang et al., 2012; Marcinkowska-Lesiak et al., 2016). Therefore, the higher shear force value in WB was due to the low protein breakdown of the broiler that is induced by less stress (Kim et al., 2020). Meanwhile, the shear force values of CB and WB decreased with increased cold storage time ($P < 0.05$). The tenderness increased during postmortem aging in chilling conditions by enzymatic degradation of the myofibrillar structural proteins (Fu et al., 2015).

In summary, most of the quality characteristics (pH, L^*- and b^*-value, and shear force) were affected by stress-inducing conditions during rearing, and CB and WB showed significantly different results. Similarly, an increase in cold storage days influenced some physicochemical attributes (pH, b^* value, cooking loss, and shear force) of both CB and WB. Among the physicochemical traits, only the L^* value showed an interaction between main effects (farm condition and storage day; Table S2). Furthermore, the physicochemical traits, except for cooking loss and WHC, remained constant with an increase in storage days. This suggests that consumers may not observe the changes in chicken breast until 7 d when stored under proper refrigeration conditions.

Storage Stability

The storage stabilities of CB and WB showed similar results throughout the storage period. In the case of total aerobic bacteria, no significant difference was found between CB and WB during the storage period

Table 3. Storage stability of chicken breast meats from conventional and animal welfare farms during cold storage.

| Item | Treatment | Storage (days) | | | | SEM[1] |
		1	3	5	7	
Total aerobic	CB	2.84[c]	3.51[b]	3.60[b]	4.35[a]	0.161
bacteria	WB	2.68[c]	3.38[bc]	3.68[ab]	4.38[a]	0.183
(log CFU/g)	SEM[2]	0.164	0.235	0.149	0.120	
TBARS	CB	0.08[b]	0.11[ab]	0.11[ab]	0.14[a]	0.009
(mg MDA/kg)	WB	0.09[c]	0.11[bc]	0.13[ab]	0.15[a]	0.008
	SEM[2]	0.030	0.010	0.008	0.011	
VBN	CB	10.73[b]	12.34[a]	12.66[a]	12.80[a]	0.296
(mg/100 g)	WB	10.97[b]	11.62[b]	12.48[a]	12.46[a]	0.200
	SEM[2]	0.271	0.264	0.166	0.291	

Abbreviations: CB, conventional farm reared-broilers; MDA, malondialdehyde; TBARS, 2-thiobarbituric acid reactive substance; VBN, volatile basic nitrogen; WB, welfare farm reared-broilers.

[a-c]Different letters within the same row differ significantly ($P < 0.05$).
[1]Standard error of mean (n = 20).
[2]Standard error of mean (n = 10).

(Table 3), and the bacterial populations in both CB and WB increased with an increase in the storage period ($P < 0.05$). This supports the reason for the changes in pH during storage. Sallam and Samejima (2004) observed that aerobic plate counts of chicken breast muscle gradually increased during 12 d of chilled storage time with an increase in the pH. *E. coli* coliforms were not detected in all samples and storage days in the present study (data not shown).

TBARS and VBN are good indicators of lipid oxidation and spoilage, respectively (Lee et al., 2018). In the present study, the TBARS and VBN values of chicken breast gradually increased with increasing storage time ($P < 0.05$), whereas there was no significant difference between CB and WB (Table 3). In our preliminary study, no significant differences were found in the antioxidant activity of between CB and WB from the results of the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid radical scavenging activity, ferric reducing antioxidant power, and oxygen radical absorption capacity (Table S3). The low-fat content and heme pigment in chicken breast may contribute to minor changes in TBARS of CB and WB (Min et al., 2008; Peiretti et al., 2011).

Considering the results from storage stability, similar properties were observed between CB and WB in terms of quality deterioration during cold storage. The breast meat from both CB and WB lost storage stability during storage, resulting in a gradual deterioration in meat quality.

Metabolomic Analysis on Meat Quality

Multivariate Analysis PLS-DA was used to visualize the discrimination between CB and WB for each storage day (Figure 1). The chicken breast meat samples from the two different rearing conditions were clearly distinguished from each other on all storage days. From these results, it can be inferred that rearing systems can affect metabolic changes in chickens. Consequently, CB and
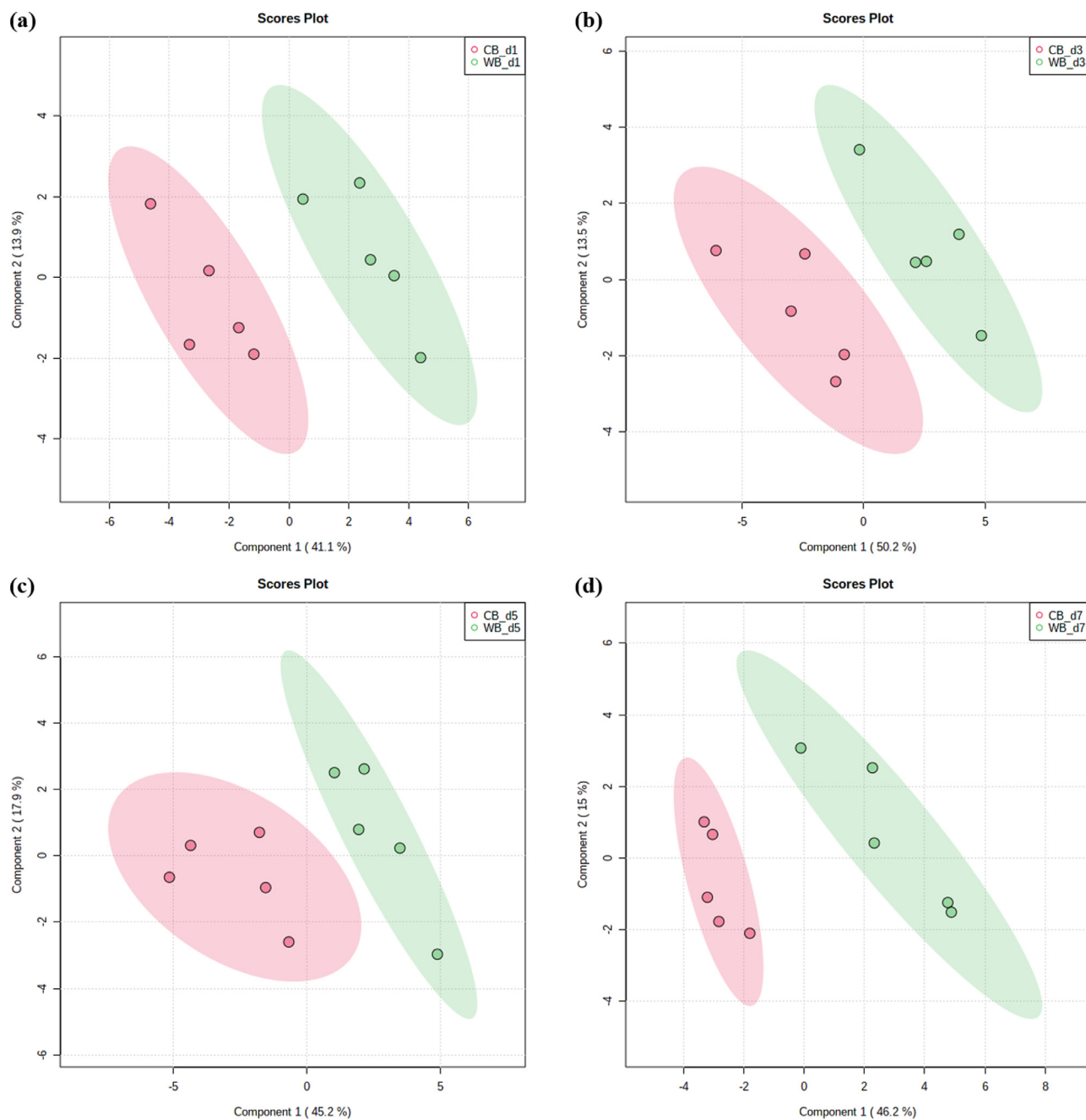
Figure 1. Partial least squares-discriminant analysis (PLS-DA) of the breast meat from broilers that were reared conventional (CB) and animal welfare farms (WB) after each cold-storage day. (A) d 1; (B) d 3; (C) d 5; (D) d 7.

WB showed distinct metabolites in breast meat during storage. If the compounds can be identified, which are influenced by the metabolic changes in chickens with different rearing environments, they can be used as potential biomarkers for differentiation. For example, Tian et al. (2015) reported that 13 metabolites related to carbohydrate, amino acid, and lipid metabolism are likely to be potential biomarkers for diagnosing heat stress status in dairy cows. Further details about the metabolites that were ascribed to different stress levels from rearing environments are discussed the section below.

Metabolite Differences Between Farm Conditions
Using ^{1}H NMR analysis, 25 metabolites were quantitatively identified in CB and WB during cold storage for 7

d (Table 4). Among them, 15 compounds (acetate, alanine, anserine, glucose, glutamate, glycine, isoleucine, lactate, leucine, phenylalanine, tyrosine, uridine monophosphate, uracil, valine, and β-alanine) were significantly different between CB and WB with a large proportion of free amino acids. In addition, the variable importance in projection (**VIP**) score was calculated, which expresses the importance of the variables for the discrimination of groups in PLS-DA. Metabolites with VIP scores higher than 1.0 were considered to be the major contributors to the formation of the PLS-DA model (Kim et al., 2021b). In our study, the VIP scores were calculated to evaluate the importance of individual metabolites in separating CB and WB in PLS-DA and how they changed during 7 d of refrigerated storage. As

Table 4. NMR-based metabolites (mg/100 g) of chicken breast meats from conventional and animal welfare farms and cold storage.

	Farm			Storage (days)					P value	
Item	CB	WB	SEM[1]	1	3	5	7	SEM[1]	Farm	Storage
Acetate	4.38[a]	3.65[b]	0.159	3.31[b]	3.89[ab]	4.33[a]	4.54[a]	0.209	**	***
Alanine	39.92[a]	32.38[b]	1.734	26.82[c]	34.20[b]	40.09[ab]	43.48[a]	1.865	***	***
Anserine	378.97[b]	439.60[a]	9.128	406.88	424.23	392.36	413.67	16.223	***	ns
Aspartate	25.11	24.20	1.461	15.30[c]	25.10[b]	27.12[b]	31.10[a]	0.878	ns	***
Creatine	376.97	368.14	5.016	377.39	380.16	370.09	362.59	7.079	ns	ns
Ethanol	1.54	1.36	0.084	1.34	1.67	1.36	1.43	0.118	ns	ns
Fumarate	0.10	0.11	0.017	0.06	0.11	0.13	0.12	0.023	ns	ns
Glucose	16.52[b]	21.13[a]	1.554	24.32[a]	19.11[ab]	16.73[ab]	15.14[b]	2.085	*	*
Glutamate	37.76[a]	29.48[b]	1.851	24.51[c]	30.53[bc]	37.44[ab]	42.00[a]	2.052	***	***
Glycine	46.97[a]	34.73[b]	1.677	32.89[b]	39.47[ab]	45.78[a]	45.27[a]	2.659	***	***
Hypoxanthine	15.55	13.74	0.968	10.88[c]	13.66[bc]	15.69[ab]	18.34[a]	1.113	ns	**
IMP	129.33	131.36	5.645	160.38[a]	134.27[b]	118.25[bc]	108.47[c]	4.953	ns	***
Inosine	74.14	71.12	2.722	58.77[b]	73.76[a]	78.46[a]	79.52[a]	2.876	ns	***
Isoleucine	13.11[a]	9.38[b]	0.773	6.76[c]	10.94[b]	12.67[ab]	14.59[a]	0.848	***	***
Lactate	639.38[b]	703.08[a]	10.255	671.85	682.92	666.73	663.44	18.129	**	ns
Leucine	11.66[b]	9.10[b]	0.626	6.64[c]	10.18[b]	11.53[ab]	13.19[a]	0.603	***	***
Methylmalonate	7.01	7.28	0.116	6.78	7.21	7.30	7.28	0.159	ns	ns
NAD^{+}	11.55	10.28	0.490	13.61[a]	11.34[b]	9.89[bc]	8.84[c]	0.443	ns	***
Niacinamide	7.48	7.44	0.110	7.10	7.59	7.50	7.64	0.142	ns	ns
Phenylalanine	11.34[a]	8.94[b]	0.579	6.83[c]	9.83[b]	11.03[ab]	12.88[a]	0.576	***	***
Tyrosine	20.61[a]	17.42[b]	0.724	15.18[b]	19.06[a]	19.76[a]	22.06[a]	0.840	***	***
UMP	2.72[a]	2.06[b]	0.190	3.31[a]	2.39[b]	1.95[b]	1.92[b]	0.231	**	***
Uracil	1.25[a]	0.50[b]	0.120	0.81	0.68	0.88	1.134	0.207	**	ns
Valine	15.50[a]	11.53[b]	0.896	8.22[c]	13.14[b]	14.98[ab]	17.73[a]	0.892	***	***
β-alanine	35.64[a]	22.25[b]	1.941	27.10	29.82	32.48	26.39	3.506	***	ns

Abbreviations: CB, conventional farm reared-broilers; WB, welfare farm reared-broilers.

[a-c]Different letters within the same row differ significantly ($P < 0.05$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant ($P > 0.05$).

[1]Standard error of mean (n = 40).

a result, the metabolites with a VIP score higher than 1.0 differed slightly among storage days, and these differences seem to induce distinction in meat quality between CB and WB. Six metabolites, namely glycine, isoleucine, leucine, phenylalanine, valine, and β-alanine,

Table 5. List of metabolites with variable importance in projection (VIP) score higher than 1.0 between breast meat broilers that were reared from conventional (CB) and animal welfare farms (WB) for each storage day.

	Storage (days)			
Item	1	3	5	7
Acetate	O			O
Alanine	O	O		O
Anserine	O	O		O
Aspartate				
Creatine				
Ethanol	O	O		
Fumarate				
Glucose		O		
Glutamate		O	O	O
Glycine	O	O	O	O
Hypoxanthine	O			
IMP				
Inosine				
Isoleucine	O	O	O	O
Lactic acid	O	O		
Leucine	O	O	O	O
Methylmalonate	O			
NAD			O	
Niacinamide				
Phenylalanine	O	O	O	O
Tyrosine	O	O		O
UMP			O	O
Uracil		O		O
Valine	O	O	O	O
β-alanine	O	O	O	O

O, included as a metabolite with VIP score >1.0 on that day.

were identified with a VIP score >1.0 on all storage days (Table 5), which may play a key role in the formation of the PLS-DA model regardless of the change in storage period. As discussed above, these 6 free amino acids produced via higher protein degradation levels in CB than WB ($P < 0.05$) on all refrigerated storage days caused differences in quality properties such as higher L^* value and lower shear force in CB (Figure 2, Table 4). When a chicken is under stress, the absorption of energy substances decreases and body energy expenditure increases, leading to insufficient energy supply (Ma et al., 2021). The hypothalamic-pituitary-adrenal axis is then activated to increase the concentration of circulating corticosterone hormone, which increases the rate of conversion from meat protein to amino acid by suppressing protein synthesis and promoting protein breakdown (Sapolsky et al., 2000; Zhang et al., 2012). In our study, the content of most free amino acids (alanine, glutamate, glycine, isoleucine, leucine, phenylalanine, tyrosine, valine, and β-alanine) was significantly higher in CB than in WB. Considering that CB was exposed to a more stressful rearing environment, more proteolysis might have occurred in CB than in WB. Among them, some compounds (alanine, glutamate, glycine, isoleucine, phenylalanine, tyrosine, and valine) are glucogenic amino acids involved in glucose production (Newsholme et al., 2011). Hence, these 7 free amino acids might be stored in higher levels in the skeletal muscle to be used as a substrate for gluconeogenesis in CB than WB, which is related to a more stressed environment (Mayes and Rodwell, 2003). Furthermore, in a stressful situation, the amino group of branched-chain amino acids (isoleucine, leucine, and valine),
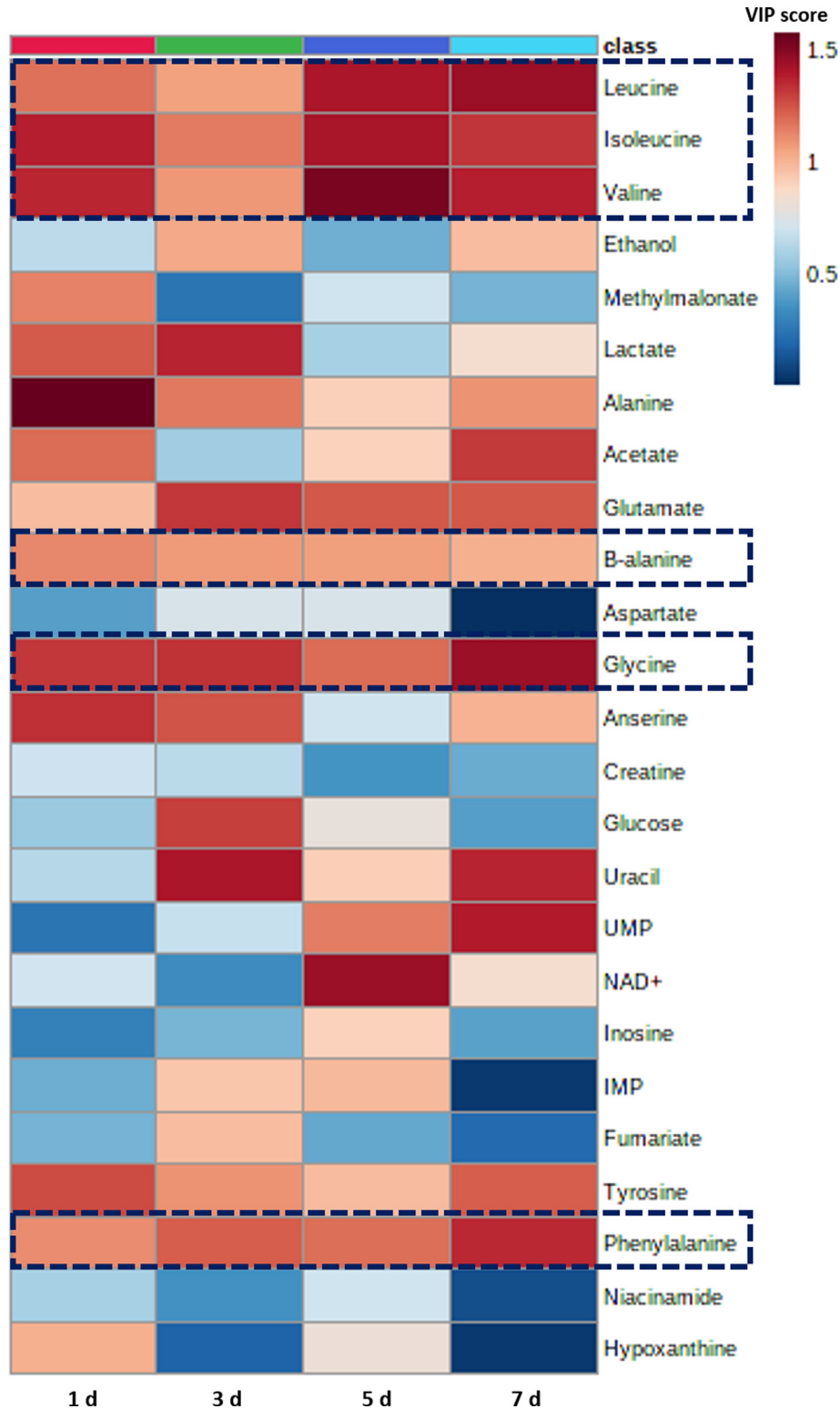
Figure 2. Heatmap for variable importance in projection (VIP) scores of the identified metabolites in breast meat from broilers that were reared conventional (CB) and animal welfare farms (WB) during cold storage. The compounds enclosed in a dotted-square had a VIP score >1.0 throughout the cold-storage period.

decomposed from the skeletal muscle of chickens, can play a role as a nitrogen donor (Palmer et al., 1985; Tinker et al., 1986). It can then produce alanine through a transamination reaction with pyruvate, which is produced through metabolic pathways such as glycogenolysis and glycolysis in muscle. Subsequently, alanine can be transferred to the liver through the blood and be

regenerated into glucose, using the carbon backbone left by the deamination reaction (DeFronzo and Felig, 1980). After glucose is transferred through the blood to the muscle, it can be utilized to produce energy. Hence, it is believed that more branched-chain amino acids (isoleucine, leucine, and valine) and alanine were accumulated in CB muscle than in WB to increase glucose supply.
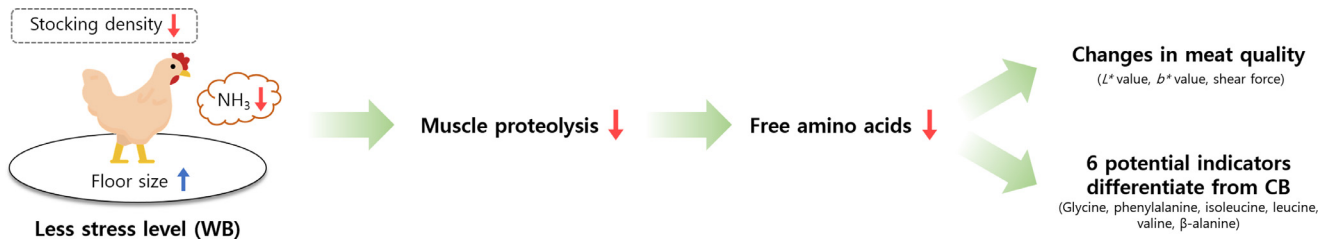
Figure 3. Overview of the changes that occurred in the broilers breast meat under animal welfare farms (WB) rearing condition.

This result corresponds with that in which the glucose content was significantly lower in CB.

The glucose and lactate contents were significantly higher in WB than in CB. This result was attributed to WB reared in a relatively less stressful condition than CB (Castellini et al., 2002). As we confirmed above, this result supports that of pH, which was lower in WB because the broilers raised on welfare farms accumulated higher levels of glycogen before slaughter. Both glucose and lactate had VIP scores >1 only on the initial storage days. Likewise, the pH value corresponds to lactate, and there was also a difference in pH between CB and WB in the early stage of storage. However, the lactate content of CB and WB gradually decreased; eventually, there was no significant difference in pH due to the rearing effect on d 7. Therefore, it is inferred that lactate on the initial storage day was linked to the gap in pH values between CB and WB. Recent research has found that glucose and lactate, which are glycolytic metabolites, are highly positively correlated with the concentration of anserine in chicken muscle (Baldi et al., 2021). These authors explained that anserine in chicken muscle acts as an endogenous buffer, preventing a rapid drop in pH postmortem. In the current study, the anserine content was higher in WB, which had higher concentrations of glycolytic metabolites (glucose and lactate) than CB (P < 0.0001). In addition, anserine is a histidine dipeptide that is abundant in non-mammalian skeletal muscles, such as poultry, and is a well-known bioactive compound with therapeutic activity (Jung et al., 2013). It is also related to umami flavor (Dashdorj et al., 2015). Consistent with our findings, Kim et al. (2020) reported that anserine content was higher in breast meat from animal welfare farm than that from conventional farm. In summary, WB, presumably less exposed to a stressful environment than CB, stored more anserine and glycolytic metabolites in breast muscle.

The effect of animal-friendly rearing on meat quality and metabolites is summarized in Figure 3 when compared with conventional rearing systems. In the WB broiler, the reduced stress levels resulted in decreased muscle proteolysis, leading to a decrease in the content of several metabolites, mainly free amino acids. These metabolic changes in WB resulted in decreased L^* and b^* values and increased shear force. Thus, the 6 metabolites (glycine, isoleucine, leucine, phenylalanine, valine, and β-alanine) with significantly different concentration and a VIP score >1 between CB and WB can be regarded as potential indicators that separate the animal-friendly reared broilers from the conventional ones.

Metabolite Differences During Refrigerated Storage

Overall, the free amino acid (alanine, aspartic acid, glutamate, glycine, isoleucine, leucine, phenylalanine, tyrosine, and valine), acetic acid, hypoxanthine, and inosine contents were significantly increased, whereas those of glucose, inosine-5'-monophosphate (**IMP**), NAD$^+$, and uridine monophosphate (**UMP**) were decreased in both CB and WB with increase in the number of storage days (P < 0.05, Table 4). Fresh meat can be spoiled by proteolysis and microbial growth during refrigeration, and various peptides and free amino acids are produced by protein degradation (Triki et al., 2018). Moreover, acetic acid can be produced during meat fermentation by lactic acid bacteria, and glucose can be utilized by microorganisms in chicken meat for their growth (Shukla et al., 2015; Mansur et al., 2019). Therefore, the concentrations of these metabolites in CB and WB were closely related to microbial growth and were affected by chilled storage, among which free amino acids and acetate increased and glucose decreased (P < 0.05).

The content of some nucleotide-related products (hypoxanthine, IMP, inosine, and UMP) in chicken breast was also affected by refrigerated storage. As enzymatic reaction occurs after slaughter, IMP breaks down, with the simultaneous accumulation of inosine and hypoxanthine in fresh meat (Kim et al., 2021b). Similarly, UMP can also be decomposed into nucleosides and nucleobases by the catalytic reaction of 5'-nucleotidase during cold storage (Dong et al., 2020). Previous studies have reported that these nucleotide-related compounds may be used to estimate meat freshness (Parris et al., 1983; Zhang et al., 2020). Therefore, it is suggested that the contents of the four nucleotide-related compounds were significantly affected by the change in cold storage day due to the degradation of metabolites by enzymatic activation in CB and WB.

NAD$^+$ is a cofactor that participates in oxidation-reduction reactions. It is known to regulate various metabolic pathways, such as glycolysis, the TCA cycle, and fatty acid oxidation (Xie et al., 2020). Based on our results, it is inferred that the decrease in NAD$^+$ in chicken breast during cold storage is due to postmortem glycolysis, as two NAD$^+$ molecules are consumed to generate one glucose, which is further converted to pyruvate (Cantó et al., 2015).

CONCLUSIONS

This study aimed to investigate the effect of an animal-friendly rearing system on the breast meat quality

and metabolomic profile of broilers in aerobic cold storage. The metabolism of chicken breast is affected by rearing systems exposed to different levels of stress. CB and WB showed different metabolite features; particularly, the content of free amino acids was significantly higher in CB. In this regard, it is considered that the metabolite profiles of CB and WB were majorly affected by the muscle proteolysis due to different stress levels. The differences in some physicochemical characteristics of CB and WB were significantly affected by differences in their metabolomic profiles. In the present study, 6 free amino acids (glycine, phenylalanine, isoleucine, leucine, valine, and β-alanine) could be recognized as candidates for indicators to distinguish animal-friendly reared chickens from conventional ones. Furthermore, future studies on serum metabolites would be helpful in broadening the library of potential markers that have an impact on more metabolic pathways affected by rearing conditions.

ACKNOWLEDGMENTS

DISCLOSURES

The authors declare that they have no conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j. psj.2022.101953.

REFERENCES

An, Y. S., J. G. Park, I. S. Jang, S. H. Sohn, and Y. S. Moon. 2012. Effects of high stocking density on the expressions of stress and lipid metabolism associated genes in the liver of chicken. J. Life Sci. 22:1672–1679.

Baldi, G., F. Soglia, L. Laghi, A. Meluzzi, and M. Petracci. 2021. The role of histidine dipeptides on postmortem acidification of broiler muscles with different energy metabolism. Poult. Sci. 100:1299–1307.

Barbut, S. 1993. Colour measurements for evaluating the pale soft exudative (PSE) occurrence in turkey meat. Food Res. Int. 26:39–43.

Cantó, C., K. J. Menzies, and J. Auwerx. 2015. NAD$^+$ metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. Cell Metab 22:31–53.

Castellini, C., C. Mugnai, and A. Dal Bosco. 2002. Effect of organic production system on broiler carcass and meat quality. Meat Sci 60:219–225.

da Rosa, P. P., B. P. Ávila, I. Damé Veber Angelo, R. G. Chesini, T. A. Fernandes, J. da Silva Camacho, M. Bugoni, V. F. B. Roll, and M. A Gularte. 2021. Impact of different chicken meat production systems on consumers' purchase perception. Br. Poult. Sci. 62:387–395.

Dashdorj, D., T. Amna, and I. Hwang. 2015. Influence of specific taste-active components on meat flavor as affected by intrinsic and extrinsic factors: an overview. Eur. Food Res. Technol. 241:157–171.

da Silva, D. C. F., A. M. V. de Arruda, and A. A Gonçalves. 2017. Quality characteristics of broiler chicken meat from free-range and industrial poultry system for the consumers. J. Food Sci. Tech. 54:1818–1826.

DeFronzo, R. A., and P. Felig. 1980. Amino acid metabolism in uremia: insights gained from normal and diabetic man. Am J. Clin. Nutr. 33:1378–1386.

Dodge, J. W., and W. J. Stadelman. 1960. Studies on tenderness evaluation. Poult. Sci. 39:184–187.

Dong, M., L. Qin, L. X. Ma, Z. Y. Zhao, M. Du, K. Kunihiko, and B. W. Zhu. 2020. Postmortem nucleotide degradation in turbot mince during chill and partial freezing storage. Food Chem 311:125900.

Enfält, A. C., K. Lundström, I. Hansson, N. Lundeheim, and P. E. Nyström. 1997. Effects of outdoor rearing and sire breed (Duroc or Yorkshire) on carcass composition and sensory and technological meat quality. Meat Sci 45:1–15.

Faustman, C., and S. P. Suman. 2017. The eating quality of meat - I Color. Pages 329−356 in Lawrie's Meat Science. F. Toldrá, ed. Woodhead publishing, Duxford, UK.

Fu, Q. Q., R. Liu, W. G. Zhang, Y. P. Li, J. Wang, and G. H. Zhou. 2015. Effects of different packaging systems on beef tenderness through protein modifications. Food Bioprocess. Tech. 8:580–588.

Henchion, M., M. McCarthy, V. C. Resconi, and D. Troy. 2014. Meat consumption: trends and quality matters. Meat Sci 98:561–568.

Hughes, J., S. Oiseth, P. Purslow, and R. Warner. 2014. A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. Meat Sci 98:520–532.

Jang, A. R., J. S. Ham, D. W. Kim, H. S. Chae, D. W. Kim, S. H. Kim, K. H. Seol, M. H. Oh, and D. H Kim. 2011. Effect of quercetin and methoxylated quercetin on chicken thigh meat quality during cold storage. Korean J. Poult. Sci. 38:265–273.

Jung, S., Y. S. Bae, H. J. Kim, D. D. Jayasena, J. H. Lee, H. B. Park, K. N. Heo, and C. Jo. 2013. Carnosine, anserine, creatine, and inosine 5′-monophosphate contents in breast and thigh meats from 5 lines of Korean native chicken. Poult. Sci. 92:3275–3282.

Jung, S., J. H. Choe, B. Kim, H. Yun, Z. A. Kruk, and C. Jo. 2010. Effect of dietary mixture of gallic acid and linoleic acid on antioxidative potential and quality of breast meat from broilers. Meat Sci 86:520–526.

Karunanayaka, D. S., D. D. Jayasena, and C. Jo. 2016. Prevalence of pale, soft, and exudative (PSE) condition in chicken meat used for commercial meat processing and its effect on roasted chicken breast. J. Anim. Sci. Technol. 58:1–8.

Kim, H. C., Y. J. Ko, M. Kim, J. Choe, H. I. Yong, and C. Jo. 2019. Optimization of 1D ^{1}H quantitative NMR (nuclear magnetic resonance) conditions for polar metabolites in meat. Food Sci. Anim. Resour. 39:1–12.

Kim, H. C., Y. J. Ko, and C. Jo. 2021a. Potential of 2D qNMR spectroscopy for distinguishing chicken breeds based on the metabolic differences. Food Chem 342:128316.

Kim, H. C., D. G. Yim, J. W. Kim, D. Lee, and C. Jo. 2021b. Nuclear magnetic resonance (NMR)-based quantification on flavor-active and bioactive compounds and application for distinguishment of chicken breeds. Food Sci. Anim. Resour. 41:312–323.

Kim, H. E., J. R. Park, J. E. Lee, D. R. Kang, A. R. Jang, H. S. Choe, and K. S. Shim. 2021c. Comparison of blood gas, biochemical factors, and hormone concentrations, and muscle stress response factors of chickens in general farms and animal welfare farms. Korean J. Org. Agric. 29:415–427.

Kim, H. J., J. J. Jeon, H. S. Kim, J. Son, K. Y. Kim, A. S. You, E. C. Hong, B. S. Kang, and H. K. Kang. 2021d. Effects of stocking

density on the growth performance, immune status and breast meat quality of broiler. Korean J. Poult. Sci. 48:13–22.

Kim, H. J., D. Kim, H. J. Kim, J. S. Kwon, and A. Jang. 2021e. Physicochemical characteristics and bioactive compounds of thigh meat from Cobb broiler in animal welfare farm. Food Life 2021:67–78.

Kim, H. J., H. J. Kim, J. Jeon, K. C. Nam, K. S. Shim, J. H. Jung, K. S. Kim, Y. Choi, S. H. Kim, and A. Jang. 2020. Comparison of the quality characteristics of chicken breast meat from conventional and animal welfare farms under refrigerated storage. Poult. Sci. 99:1788–1796.

Kim, Y. H. B., R. D. Warner, and K. Rosenvold. 2014. Influence of high pre-rigor temperature and fast pH fall on muscle proteins and meat quality: a review. Anim. Prod. Sci. 54:375–395.

Lee, H. J., J. Choe, J. W. Yoon, S. Kim, H. Oh, Y. Yoon, and C. Jo. 2018. Determination of salable shelf-life for wrap-packaged dry-aged beef during cold storage. Korean J. Food Sci. Anim. Resour. 38:251–258.

Lin, C. C., and C. S. Lin. 2005. Enhancement of the storage quality of frozen bonito fillets by glazing with tea extracts. Food Control 16:169–175.

Lin, C. Y., H. Y. Kuo, and T. C. Wan. 2014. Effect of free-range rearing on meat composition, physical properties and sensory evaluation in Taiwan game hens. Asian Australas. J. Anim. Sci. 27:880–885.

Ma, B., L. Zhang, J. Li, T. Xing, Y. Jiang, and F. Gao. 2021. Heat stress alters muscle protein and amino acid metabolism and accelerates liver gluconeogenesis for energy supply in broilers. Poult. Sci. 100:215–223.

MAFRA. 2012. Enriched animal welfare farm assurance program. Ministry of Agriculture, Food and Rural Affairs, Korea.

Mansur, A. R., D. H. Seo, E. J. Song, N. E. Song, S. H. Hwang, M. Yoo, and T. G. Nam. 2019. Identifying potential spoilage markers in beef stored in chilled air or vacuum packaging by HS-SPME-GC-TOF/MS coupled with multivariate analysis. LWT Food Sci. Technol. 112:108256.

Marcinkowska-Lesiak, M., Ż. Zdanowska-Sąsiadek, A. Stelmasiak, K. Damaziak, M. Michalczuk, E. Połtawska, J. Wyrwisz, and A. Wierzbicka. 2016. Effect of packaging method and cold-storage time on chicken meat quality. CYTA. J. Food 14:41–46.

Min, B., K. C. Nam, J. Cordray, and D. U. Ahn. 2008. Endogenous factors affecting oxidative stability of beef loin, pork loin, and chicken breast and thigh meats. J. Food. Sci. 73:439–446.

Muroya, S., S. Ueda, T. Komatsu, T. Miyakawa, and P. Ertbjerg. 2020. MEATabolomics: muscle and meat metabolomics in domestic animals. Metabolites 10:188.

Mayes, P. A., and V. W. Rodwell. 2003. Overview of metabolism. Pages 122−129 in Harper's Illustrated Biochemistry. R. K. Murrary, D. K. Granner, P. A. Mayes and V. W. Rodwell, eds. 26th ed.. McGraw-Hill Education, New York, NY.

Newsholme, P., L. Stenson, M. Sulvucci, R. Sumayao, and M. Krause. 2011. Amino acid metabolism. Pages 3−14 in Comprehensive Biotechnology. M. Moo-Young, ed. 2nd ed. Elsevier, UK.

Nychas, G. J. E., P. N. Skandamis, C. C. Tassou, and K. P. Koutsoumanis. 2008. Meat spoilage during distribution. Meat Sci 78:77–89.

Oswell, N. J., O. P. Gilstrap, and R. B. Pegg. 2021. Variation in the terminology and methodologies applied to the analysis of water holding capacity in meat research. Meat Sci 178:108510.

Palmer, T. N., M. A. Caldecourt, K. Snell, and M. C. Sugden. 1985. Alanine and inter-organ relationships in branched-chain amino and 2-oxo acid metabolism. Biosci. Rep. 5:1015–1033.

Parris, N., S. A. Palumbo, and T. J. Montville. 1983. Evaluation of inosine monophosphate and hypoxanthine as indicators of bacterial growth in stored red meat. J. Food Prot. 46:614–617.

Peiretti, P. G., C. Medana, S. Visentin, V. Giancotti, V. Zunino, and G. Meineri. 2011. Determination of carnosine, anserine, homocarnosine, pentosidine and thiobarbituric acid reactive substances

contents in meat from different animal species. Food Chem 126:1939–1947.

Petracci, M., S. Mudalal, E. Babini, and C. Cavani. 2014. Effect of white striping on chemical composition and nutritional value of chicken breast meat. Ital. J. Anim. Sci. 13:179–183.

Ponte, P. I. P., C. C. Rosado, J. P. Crespo, D. G. Crespo, J. L. Mourão, M. A. Chaveiro-Soares, J. L. A. Brás, I. Mendes, L. T. Gama, J. A. M. Prates, L. M. A. Ferreira, and C. M. G. A Fontes. 2008. Pasture intake improves the performance and meat sensory attributes of free-range broilers. Poult. Sci. 87:71–79.

Sallam, K. I., and K. Samejima. 2004. Effects of trisodium phosphate and sodium chloride dipping on the microbial quality and shelf life of refrigerated tray-packaged chicken breasts. Food Sci. Biotechnol. 13:425.

Sapolsky, R. M., L. M Romero, and A. U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21:55–89.

Shin, D. J., D. G. Yim, J. A. Kwon, S. S. Kim, H. J. Lee, and C. Jo. 2021. Effect of cutting time and cooking temperature on physicochemical properties of chicken breast meat emulsion sausage with olive oil. Poult. Sci. 101:101554.

Shukla, V., G. Kandeepan, and M. R. Vishnuraj. 2015. Evaluation of shelf life of buffalo meat in aerobic cold storage using physicochemical parameters. Buffalo Bull 34:453–457.

Tian, H., W. Wang, N. Zheng, J. Cheng, S. Li, Y. Zhang, and J. Wang. 2015. Identification of diagnostic biomarkers and metabolic pathway shifts of heat-stressed lactating dairy cows. J. Proteomics 125:17–28.

Tinker, D. A., J. T. Brosnan, and G. R. Herzberg. 1986. Interorgan metabolism of amino acids, glucose, lactate, glycerol and uric acid in the domestic fowl (*Gallus domesticus*). Biochem. J. 240:829–836.

Triki, M., A. M. Herrero, F. Jiménez-Colmenero, and C. Ruiz-Capillas. 2018. Quality assessment of fresh meat from several species based on free amino acid and biogenic amine contents during chilled storage. Foods 7:132.

Troy, D. J., and J. Kerry. 2010. Consumer perception and the role of science in the meat industry. Meat Sci 86:214–226.

Warner, R. D. 2017. The eating quality of meat - IV Water-holding capacity and juiciness. Pages 419−459 in Lawrie's Meat Science. F. Toldrá, ed. Woodhead publishing, Duxford, UK.

Wen, D., Y. Liu, and Q. Yu. 2020. Metabolomic approach to measuring quality of chilled chicken meat during storage. Poult. Sci. 99:2543–2554.

Xie, N., L. Zhang, W. Gao, C. Huang, P. E. Huber, X. Zhou, C. Li, G. Shen, and B. Zou. 2020. NAD$^+$ metabolism: pathophysiologic mechanisms and therapeutic potential. Signal Transduct. Target. Ther. 5:1–37.

Xing, T., F. Gao, R. K. Tume, G. Zhou, and X. Xu. 2019. Stress effects on meat quality: a mechanistic perspective. Compr. Rev. Food Sci. Food Saf. 18:380–401.

Zaboli, G., X. Huang, X. Feng, and D. U. Ahn. 2019. How can heat stress affect chicken meat quality?−a review. Poult. Sci. 98:1551–1556.

Zhang, Z., G. Jia, J. Zuo, Y. Zhang, J. Lei, L. Ren, and D. Feng. 2012. Effects of constant and cyclic heat stress on muscle metabolism and meat quality of broiler breast fillet and thigh meat. Poult. Sci. 91:2931–2937.

Zhang, T., S. Zhang, L. Chen, H. Ding, P. Wu, G. Zhang, K. Xie, G. Dai, and J. Wang. 2020. UHPLC−MS/MS-based nontargeted metabolomics analysis reveals biomarkers related to the freshness of chilled chicken. Foods 9:1326.

Zhang, W. H., F. Gao, Q. F. Zhu, C. Li, Y. Jiang, S. F. Dai, and G. H. Zhou. 2011. Dietary sodium butyrate alleviates the oxidative stress induced by corticosterone exposure and improves meat quality in broiler chickens. Poult. Sci. 90:2592–2599.